*Contributions to the Histo-Chemistry of Nerve: On the Nature of Wallerian Degeneration.**

By Henry O. Feiss and W. Cramer.

(Communicated by Prof. E. A. Schäfer, F.R.S. Received October 22, 1912,—Read January 23, 1913.)

(From the Physiology Department, University of Edinburgh.)

[Plate 4.]

Part I.—Introductory.

When the continuity of a medullated nerve fibre is broken the peripheral segment undergoes a series of morphological changes which are grouped together under the term "Wallerian degeneration." These changes affect all the constituent parts of the fibre, namely, the axis-cylinder, the myelin sheath and the sheath of Schwann. In the early stages they consist of fragmentation of the axis-cylinder, breaking-up of the myelin sheath, and multiplication of the nuclei of the sheath of Schwann together with an increase of protoplasm around them. Although these changes have been studied by numerous observers, and although there is a general agreement with regard to their morphological appearances, different interpretations have been put upon them. And it is not difficult to understand why that should be so.

The process of degeneration is intimately related to the process of regeneration, and the differences which exist in the interpretation of the latter process are reflected to some extent in the interpretations of the phenomena of degeneration.

The degenerative changes in the axis-cylinder and medullary sheath are explained by some authors as being due to the separation of the protoplasm of the nerve fibre from the nerve cell, its nutritive centre, by others as the result of traumatism, while according to Ranvier (1) they are produced by the mechanical effect of the proliferating neurilemmal cells. The proliferation of these cells again has received different explanations. According to Ströbe (2), for instance, the neurilemmal cells are connective tissue cells, and their proliferation is of the nature of the connective tissue reaction, which takes place in the process of repair following the lesion of the specific parenchyma of any organ. According to other observers these cells are of nervous origin

* The expenses of this research were defrayed by a grant from the Moray Fund of the University of Edinburgh.

and they play an all important part in the process of nerve regeneration. According to this latter view (5) the phenomena of Wallerian degeneration "do not deserve the term phenomena of degeneration. They are not phenomena of degeneration or death but of reorganisation or life."

We have indicated here only some of the views which are held. But this brief survey indicates sufficiently well that the various explanations of the processes of Wallerian degeneration cover a field so wide as to include their interpretation as phenomena of death on the one hand and of exaggerated phenomena of life on the other.

Although the question which thus arises is one of considerable importance, and although it can be tested experimentally, the evidence on this point is neither ample nor convincing. The importance which Ranvier attached to the proliferation of the neurilemmal cells in the process of Wallerian degeneration led him to study the changes which take place in a nerve after the death of the organism. He found (1) that the nerve of a dead animal which was allowed to lie about "somewhere in a corner of the laboratory" for several days did not show in the myelin sheath anything similar to the changes of Wallerian degeneration.

The subject does not appear to have received any attention by subsequent workers until it was taken up again by Mönckeberg and Bethe (3). These authors have apparently not been aware of Ranvier's experiments, and the observations which they record do not quite agree with those of Ranvier. They found, 24 hours after the death of an animal, a certain amount of disintegration in the axis-cylinder and the sheaths of the nerves, and these changes were more advanced when the body was kept at 37° C. But these changes were not more pronounced two and three days after death than they were after the first 24 hours. Nerves removed from a living animal and kept in a moist chamber for one or two days did not show the characteristic changes of the Wallerian degeneration in the axis-cylinder and the myelin sheath. Mönckeberg and Bethe concluded from their experiments that the process of Wallerian degeneration is dependent on the life of the fibre, and, further, that it takes place only when the nerve is lying in a medium of living tissue. The degenerative changes which do occur during the first 24 hours after death are attributed to a survival of the tissues, which is stated to be prolonged by keeping the dead animal at body temperature.

Reference must be made also to the observations of Merzbacher (4), because they have an important bearing on the problem before us. From observations on nerve degeneration in hibernating animals, and on frogs kept at different temperatures, he concluded that in the living animal the occurrence of Wallerian degeneration is dependent upon the temperature.

If the body temperature is below a certain minimum, the processes of degeneration are arrested, or, at any rate, greatly retarded.

These observations invalidate to a certain extent the conclusions of Ranvier and of Mönckeberg and Bethe. They might explain why the results of Ranvier's experiments above referred to were negative, and why in the experiments of Mönckeberg and Bethe the degenerative changes in the nerves of dead animals were more advanced when the body was kept at 37° than when the temperature was allowed to fall to room temperature.

Merzbacher also records some observations on the occurrence of Wallerian degeneration after transplantation. He found that nerves, after auto-transplantation (transplantation into another part of the same animal), and after homo-transplantation (transplantation into another animal of the same species), exhibited the typical changes of Wallerian degeneration, but failed to show them after hetero-transplantation (transplantation into an animal of a different species). These observations, as the author himself states, are only of a preliminary nature, and that accounts probably for the omission to state what changes, if any, take place in a nerve after hetero-transplantation. Moreover, in a repetition of these experiments, Maccabruni (5) has shown and illustrated by figures that hetero-transplanted nerves undergo Wallerian degeneration in the same way as auto-transplanted or homo-transplanted nerves.

From the observations of Mönckeberg and Bethe, and of Merzbacher, far-reaching conclusions have been drawn by van Gehuchten (6), which can best be stated in his own words: "There is no degeneration, and there cannot be any degeneration, except where there is life; degeneration is not only a sign of life, but it is even the most striking manifestation which we can have of an actual hyperactivity of normal cell life. Nerve degeneration is a reaction of the organism; it is an actual defence against the disturbances which a given traumatism has induced in the normal functioning of its nerve fibres."

These considerations lead van Gehuchten to demand a modification of the first part of Waller's law, which says that the peripheral segment of a cut nerve undergoes degeneration. According to van Gehuchten, this law holds good only if the significance of the word degeneration is altered, and if we understand by it "a process of life, a process of reorganisation, which by itself tends to a reconstitution of the nerve fibre."

It will be seen that all the authors who have discussed this question are agreed that the process of Wallerian degeneration is dependent upon the life of the nerve fibre. But this conclusion does not necessarily follow from their observations. In almost all the experiments, two different conditions—the

life of the animal and the life of the nerve fibre—were either both operative or both excluded. It is quite conceivable, however, that the conditions which are preliminary to the occurrence of Wallerian degeneration can be established in a living animal but not in a dead animal, and that, when these conditions are established, the degenerative changes in the nerve fibre itself might proceed irrespective of the life of the fibre.

Such a possibility is mentioned here in order to indicate that the life of the animal and the life of the nerve fibre are two factors, whose influence on the process of Wallerian degeneration may have to be considered separately.

In order to obtain conclusive evidence to what extent the processes of Wallerian degeneration are dependent upon the life of the nerve-fibre, it seemed desirable to carry out observations on the behaviour of nerves removed from the body and subjected to various conditions.

Part II.—Experimental.

The nerves studied in these experiments were the sciatic or popliteal nerves of adult cats. For stains osmic acid was used for the myelin sheaths and hæmatoxylin for the nuclei. The osmic acid preparations were made by placing the nerves in a 1-per-cent. solution of osmic acid for 24 hours and then washing them for 24 hours. A small piece of the nerve was then removed for teasing, and the remainder was usually embedded in paraffin.

For studying the Marchi reaction, the specimens were placed in Müller's solution for one week, and then in a mixture of two parts of Müller's and one part of osmic acid (1 per cent.) for three or four days, these steps being carried out at 40° C.

All the experiments on living cats were done under complete chloroform anæsthesia, and with careful aseptic precautions if the animals were to be kept alive after the operation.

The composition of the Ringer's solution used was as follows:—Sodium chloride, 0·9 grm.; potassium chloride, 0·1 grm.; calcium phosphate solution (saturated), ad 100 c.c.

Group I.—*Excised Nerves Kept in Ringer's Solution at* 37° *for Various Periods.*

The right sciatic nerve of an adult cat was removed under chloroform, using very careful aseptic precautions. The nerve was divided into five pieces, each about ¼ inch long. Each piece was placed in a sterile Petri dish containing sterile Ringer's solution, and all the dishes placed in an

incubator at 37° C. The pieces were removed at the end of one, two, three, four, and six days respectively, and fixed in 1-per-cent. osmic acid as described above.

Microscopical Findings.—The one- and two-day preparations showed few changes, although in the latter some fibres were beginning to break up. The three- and four-day preparations were distinctly changed. In many of the fibres the myelin sheath was broken up and beaded, showed vacuoles and irregular clumps of granular detritus. After six days the changes were very marked (see Plate 4, fig. 1).

These changes strongly suggested the classical signs of Wallerian degeneration as seen in nerves of living animals, following interruption of continuity.

The experiment was repeated in another series, and the findings were very much the same. Nerves were also kept in Ringer's solution for longer periods, up to 16 days, in order to see whether there would be a Marchi reaction. In no case was a positive Marchi reaction obtained.

Group II.—*Comparison of Excised Nerves Kept in Ringer's Solution at 37° C., with Nerves Degenerated in the Living for Equal Lengths of Time.*

Cat α.—Under complete chloroform anæsthesia and with careful aseptic precautions the right external popliteal nerve was freed, and a piece ½ inch long excised and placed in a Petri dish with sterile Ringer's solution. The wound was now closed. The Petri dish containing the excised piece of nerve was placed in the incubator at 37° C. At the end of one day the cat was killed and a piece of the peripheral stump of the divided nerve removed and placed in 1-per-cent. osmic acid. The specimen in Ringer's solution was treated likewise, and both specimens run through for teasing and embedding.

By this means one could compare a specimen of a nerve degenerated *in vivo* with another specimen of the same nerve kept *in vitro* for the same length of time.

Cats β, γ, and δ.—Repetition of the above, except that the lengths of time between the excision and the removal of the peripheral stump ends were two, three, and four days respectively.

Microscopical Findings.—The specimens kept in Ringer's solution presented the same sort of appearance as those described under Group I. Very similar to these changes were the changes to be observed in the specimens of the same nerves which were actually degenerated in the living.

There was, however, this difference. Although the degenerated fibres were more or less broken up, and the myelin collected in elongated or round masses, the stain was usually clear and well diffused. In the specimens

kept in Ringer's solution there was more detritus, and the myelin in many of the fibres showed a lack of clearness in its staining reaction. This appearance of the myelin might be described as "flaky," while that of the fibres degenerated *in vivo* might be referred to as "laked."

Paraffin sections, made from the specimens kept in Ringer's solution, were stained with hæmatoxylin, and showed the nuclei broken up and staining badly.

The next group of experiments was carried out in order to determine the effect of temperature on the changes which nerve fibres undergo *in vitro*.

Group III.—*Comparison of Excised Nerves kept in Ringer's Solution at* 37° *and at* 5° *respectively.*

External popliteal nerves were removed aseptically. Each nerve was divided into two pieces and placed in Ringer's solution in Petri dishes. One set of dishes was placed in the incubator, the other set placed in the ice box.

At the end of four and six days the nerves were removed and placed in 1-per-cent. osmic acid.

Microscopical Findings.—The nerves kept at 5° showed the characteristic changes in the myelin sheath, but these changes were not as pronounced as those seen in nerves kept at 37° for the same lengths of time.

The next group of experiments was devised in order to test whether contact with an aqueous solution was a contributory factor in bringing about these changes in the myelin sheaths. Attempts to keep nerves in the incubator in a moist chamber for several days, so that they were hanging freely in the chamber and did not come into direct contact with the solution, did not give very satisfactory results, because the nerves dried partially. The object of this group of experiments was attained, however, by immersing the nerves in liquid paraffin.

Group IV.—*Comparison of Excised Nerves kept at* 37° *in Ringer's Solution with Excised Nerves kept in Liquid Paraffin.*

External popliteal nerve removed from an adult cat under sterile precautions, and divided into two pieces. One piece was placed in Ringer's solution. The second was placed in liquid paraffin. Both specimens kept at 37°. Pieces removed at end of six days and fixed in 1-per-cent. osmic acid.

Microscopical Findings.—The specimen kept in Ringer's solution showed the characteristic signs described above under Group I. The specimen kept in liquid paraffin consisted for the most part of apparently normal fibres.

Knowing the effects of Ringer's solution on nerve fibres, the question arose whether the fluid constituents of the blood would have the same effect. This experiment was carried out in the ice box, in order to exclude the effect of bacterial contamination.

Group V.—*Comparison of Excised Nerve kept in Ice Box for Six Days in Ringer's Solution with Excised Nerve kept in Blood Serum under Similar Conditions.*

Cat chloroformed and external popliteal nerve removed under sterile precautions. The nerve was divided into two pieces and temporarily laid in a sterile dish. The carotid artery of cat opened under sterile precautions, and some blood allowed to flow into a Petri dish. The blood was allowed to clot and the dish then tilted so as to cause the serum to flow to one side. The one piece of nerve was laid in this serum, and the other was laid in a Petri dish containing sterile Ringer's solution. Both dishes were placed in the ice box. Pieces were removed at the end of six days and fixed in 1-per-cent. osmic acid.

Microscopical Findings.—Both specimens showed the changes described under Group I. The changes were about equal in amount.

One of the essential differences between the conditions of a nerve degenerating *in vivo* and a nerve kept *in vitro* is the presence in the former of a circulatory mechanism, which not only supplies the fibre with nutriment, but in this case may also have an effect in removing products of degeneration. In order to estimate the part played by this factor it was necessary to study the changes in a nerve kept *in vivo* in the absence of any circulation. This condition was obtained by tying a nerve in two places as described in the following group.

Group VI.—*Nerves tied in Two Places in Living Cats. Comparison of Preparations made from the Segment between Ligatures with Preparations taken from Segment below Lower Ligature.*

Under complete chloroform anæsthesia external popliteal nerve freed and tied very tightly with catgut ligatures in two places $\frac{3}{4}$ inch apart.

Cat killed five days later. External popliteal nerve freed again. The portion of the nerve between the ligatures shrunken to about one-half its normal size. The portion below the lower ligature was somewhat swollen. Pieces from between the ligatures and from below the lower ligature fixed in 1-per-cent. osmic acid.

Microscopical Findings.—Preparations made from the segment below the

lower ligature showed characteristic signs of a nerve degenerated for five days, while preparations from the segment between the ligatures showed the peculiar flaky appearance of the myelin that was seen in nerves kept in Ringer's solution. The contrast in the appearance of the two preparations was very marked, although the amount of breaking up of the myelin was about equal in the two segments. (See Plate 4, figs. 2 and 3.)

The difference obtained between the two segments in this experiment suggested the possibility that the interference with the circulation might have an effect on the onset of Marchi's reaction. In five cases the external popliteal nerves were tied between the ligatures. The nerves were removed after 8, 11, 12, 13, and 14 days respectively and the Marchi reaction in the segment between the ties compared with that in the segment below the ties. It need hardly be pointed out that by this method the circulation was not cut off for the whole length of time during which degeneration took place.

The 8- and 11-day specimens showed no Marchi reaction. The 12- and 13-day specimens showed beginning Marchi reactions. In neither case was there any appreciable difference between the segments compared. The 14-day specimen showed a more positive Marchi reaction. Here again there was no appreciable difference between the segments compared.

Summary.

Cats' nerves removed from the body and kept at body temperature in Ringer's solution or in blood serum exhibit certain changes in the myelin sheath as studied in osmic acid preparations, which resemble the early changes exhibited by nerves degenerated for about equal lengths of time in the living. These changes are slowed but not inhibited by lower temperatures. In nerves kept in liquid paraffin, the changes are not seen to occur to any great extent.

There is one difference in the appearance of nerves degenerated *in vivo* from that of nerves kept *in vitro:* the broken down myelin stains less clearly in the latter condition, and thus has a flaky appearance. This same flaky staining was noted in the living when the circulation of a nerve was cut off locally.

Nerves kept in Ringer's solution *in vitro* showed no Marchi reaction and no signs of nuclear activity.

Conclusions.

In discussing the nature of the changes comprised under the term Wallerian degeneration, we must consider separately the proliferation of the neurilemmal nuclei and the fragmentation of the myelin sheath. That the former is a manifestation of life goes without saying. The fragmentation of

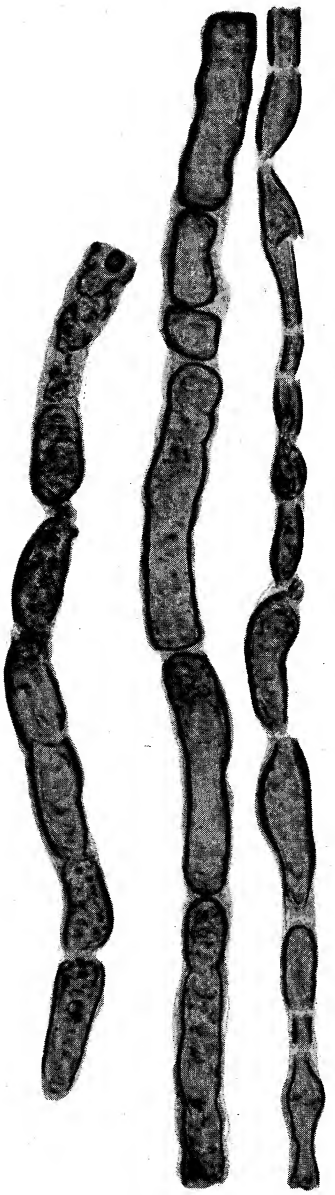

FIG. 1.

FIG. 2.

FIG. 3.

the myelin sheath, however, is not dependent upon life, since it occurs in nerves removed from the body and kept under certain conditions, in which the nuclei show no signs of proliferation.

It follows also that the changes in the myelin sheath are not dependent upon the proliferation of the neurilemmal nuclei, as has been held by some authors.

The fact that the fragmentation of the myelin sheaths in nerves kept *in vitro* is not markedly inhibited by cold indicates that the changes in the myelin sheaths are not essentially due to processes of a fermentative or autolytic nature.

On the other hand contact with an aqueous solution appears to be of essential importance in bringing about the fragmentation of the myelin sheaths in nerves kept *in vitro*. A process of imbibition appears, therefore, to be a contributory factor in bringing about the changes in the myelin sheath characteristic of Wallerian degeneration.

LITERATURE.

1. Ranvier, ' Leçons sur l'Histologie du Système Nerveux,' Paris, 1878, vol. 1, p. 289 ; vol. 2, p. 69.
2. Ströbe, ' Ziegler's Beiträge z. pathol. Anat.,' 1893, vol. 13, p. 160.
3. Mönckeberg u. Bethe, ' Archiv f. mikroskop. Anat.,' 1899, vol. 54, p. 135.
4. Merzbacher, ' Pflüger's Archiv,' 1903, vol. 100, p. 568.
5. Maccabruni, ' Folia Neuro-Biologica,' 1911, vol. 5, p. 598.
6. Van Gehuchten, ' Le Névraxe,' 1905, vol. 7, p. 203.

DESCRIPTION OF PLATE 4.

Fig. 1. Cat's nerve kept in Ringer's solution at 37° for six days. Osmic acid. Teased preparation. × 500 diam.

Fig. 2. Microphotograph of cat's nerve degenerated *in vivo*, five days after having been tied in two places. Segment below lower tie. Osmic acid. Cut in paraffin. × 200 diam.

Fig. 3. Same nerve as fig. 2. Segment between ties.

Fig. 1.

Fig. 2.

Fig. 3.